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Aberrant recombination events in B cell lines derived from a κ -deficient human

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ABSTRACT

We have analyzed the structure of Ig κ chain genes in B cell lines derived from a human individual who cannot synthesize any κ chains, and whose Igs all contain λ chains (1). We have characterized secondary DNA recombination events at two κ alleles which have undergone misaligned V-J recombinations. One such secondary recombination has joined the flanking sequences of a V κ and a J κ 2 gene segment as if it were the reciprocal product of a V-J κ 2 recombination, and resulted in the displacement of the recombined VJ κ 1 gene segments from the C κ locus. The non-rearranged form of the V κ fragment which had recombined with the J κ 2 flank was cloned. Nucleotide sequencing of this fragment identified a V κ gene that differed by at least 38% from all previously sequenced human V κ genes. The other V-J κ segment analyzed has undergone a secondary recombination at a different site from that described above, at a site within the intervening sequence between the J κ and C κ gene segments, similar to the location of secondary recombinations which have occurred in λ + B cell lines from mice and humans (2,3). These results prove that multiple recombinations can occur at one J κ -C κ locus.

INTRODUCTION

Although recombination of immunoglobulin (Ig) κ chain V and J genes results in deletion of the DNA between the recombined gene segments, V κ -J κ joining may not generally occur by an intrachromatid deletion because DNA fragments located 5' to the J κ genes, which should be deleted during intrachromatid deletion, are frequently preserved within κ -expressing hybridomas, lymphocytes and plasmacytomas (4,5). In addition, DNA fragments which appear to be reciprocal products of V κ -J κ recombination, i.e. recombined flanking regions of V κ and J κ gene segments, which would result from V-J recombination occurring by interchromatid recombination (sister chromatid exchange) or by intrachromatid inversion have been found in plasmacytomas, hybridomas and lymphomas (6-8). However, an alternative explanation for the lack of deletion of DNA sequences located 5' to the J κ gene segments and for the presence of reciprocal products of V κ -J κ

recombination is re-integration of sequences excised during intrachromatid deletion (8).

Ig C κ genes are frequently deleted from B cell lines which express Ig λ chains (9-11). Data have been presented suggesting that in mice a particular DNA sequence from the chromosome bearing the λ chain genes may be frequently involved in recombination events, apparently with a particular sequence within the intervening sequence (IVS) between the J κ and C κ gene segments, effecting the deletion of C κ genes (2). Recently it has been shown that deletion of C κ genes from human λ + cell lines is also accompanied by a recombination at an analogous position within the IVS between the J κ and C κ segments (3).

While investigating the structure of Ig κ chain genes in B cell lines derived from a human individual who cannot synthesize any κ chains and whose Igs all contain κ chains (1), we discovered and characterized a recombination event which resulted in separation of the C κ gene from a misaligned V-J κ recombination product. This event joined the 3' flank of a V κ gene with the 5' flank of J κ 2, and was the reciprocal product of a V κ -J κ 2 recombination. Unlike other examples of reciprocal products of V κ -J κ recombination which have been characterized (6-8), this recombination product is located 3' to a recombined V κ -J κ 1 gene segment. In the other cases reported, recombined flanking sequences (usually from J κ 1) were found unlinked to any recombined V-J κ segments.

The V κ gene whose flanking sequences had recombined with the J κ 2 flank was cloned and sequenced. It was found to encode a V κ domain which defines a new human V κ subgroup as it does not belong to any of the four known human V κ subgroups (12-17). This new V κ gene is a member of a single-gene family.

A second misaligned recombined V-J κ allele was also cloned, and shown to have also undergone a secondary recombination event, at a different site from that utilized in the first fragment, and which may have resulted in deletion of the C κ gene segment from the chromosome. Although the recombination junction has not been sequenced, it resides at the 5' end of the IVS between the J κ and C κ gene segments at a position analogous to that utilized in recombinations which may have resulted in the deletion of C κ genes from mouse and human λ + B cell lines (2,3).

MATERIALS AND METHODS

Establishment of lymphoblastoid cell lines (LCLs).

Sterile heparinized venous blood was obtained from a normal human adult

and from the κ -deficient patient. Leukocytes isolated according to the method of Boyum (18) were exposed to the B95-8 leukocyte transforming strain of Epstein-Barr virus (EBV) at a multiplicity of infection such that 80-100% of the wells contained transformed cells (19). LCLs were maintained at 37°C in RPMI-1640 supplemented with 17% fetal bovine serum, 1 mM glutamine, and antibiotics in an atmosphere of 5% CO₂.

Two of the LCLs from the patient (P1 and P2 in Fig. 1) were tested and found to express λ chains and λ mRNA, and no κ chains or κ mRNA (A. Solomon, Univ. of Tennessee, and M. Cooper, Univ. of Alabama, personal communications; J.S. unpublished data).

Cloning of LCLs.

0.3, 1 or 10 cells were plated into individual wells of 96 well plates, containing irradiated feeder layers of human fibroblasts. More than 20 clones from the patient's LCL and 10 clones from the normal LCL were obtained. Although the frequency of growing cells in the wells indicated that the clones originated from single cells, all 20 clones from the patient's LCL, and all 10 clones from the normal LCL were identical (by Southern blotting analyses of their J genes), but not with each other. Apparently the predominant clone in each culture had been isolated. A karyotype analysis was performed on the cloned LCL derived from the patient by Dr. P. Szabo, Cornell University, NYC. The cells contained two normal-appearing chromosome 2's (κ gene-bearing chromosome).

Genomic DNA isolation, restriction enzyme digestion, gel electrophoresis, DNA blotting.

These procedures were performed as described (20,21).

Nucleic acid annealing.

Annealing mixtures contained 50% deionized formamide, 0.02% bovine serum albumin, 0.02% polyvinyl pyrrolidine, 0.02% Ficoll, 50 μ g E.coli DNA/ml, 900 mM NaCl, 90 mM sodium citrate, 0.06 mM disodium EDTA. Annealing was performed at 42°C. The blots were washed as described (21). To remove the annealed probe so that the blots could be re-used, the blots were incubated in annealing mixture without the labeled probe at 68°C for 30 min.

Hybridization probes.

Probes were nick-translated fragments isolated from plasmids or λ phage, except as noted. The V κ probe was a 558 bp PstI fragment isolated from HK101/80, encoding a germline V κ (subgroup 1) gene segment (12, given by T. Rabbitts). The J κ , IVS, and C κ probes were fragments isolated from a recombinant λ phage containing the germline J κ -C κ gene segment (22,23, given

by P. Leder). The regions used as probes are indicated above the restriction map of the normal germline J κ -C κ gene segment in Fig. 2A. The region encoding the 1.8 kb probe derived from the recombinant phage EVJK2 is indicated beneath the restriction map of EVJK2 (Fig. 2B). A single-stranded hybridization probe complementary to the coding strand of the V κ gene present in EV15 was prepared (24) by priming DNA synthesis with a primer (PL Biochemicals) that annealed 5' to the insert in recombinant M13 phage which contained the 572 bp PstI-PvuII fragment encoding the EV15 V κ gene (Fig. 2C). Isolation of recombinant λ bacteriophage from the patient's LCLs.

a. EVJK2. DNA was prepared from an uncloned LCL (P3) which had been frozen soon after transformation, as it has been reported that normal λ +B cells are more likely to contain J κ and C κ genes than are λ +B cell lines, suggesting that J κ -C κ gene segments may be deleted from cells during growth in vitro (10,25). Bam fragments, the size of the rearranged J κ fragment in this LCL (7-12 kb) (Fig. 1B, lane P3), were purified by agarose gel electrophoresis and ligated to Bam arms of the λ vector, L47.1 (26). Three recombinant phage containing the identical insert were isolated by screening 2×10^6 plaques with the J κ probe.

b. EVJK11 and EV15. A LCL which had been passaged extensively (P1) provided the DNA used to prepare a MboI library containing 1×10^6 PFU of 14-21 kb fragments cloned in Charon 30 (27). By screening this library with the J κ probe and with the 1.8 kb probe from EVJK2, we obtained 2 recombinant phage, EVJK11 and EV15, respectively.

Cloning into M13 phage vectors and DNA sequencing.

Restriction fragments were ligated into various M13 mp vectors, and cloned by transformation of JM103 by the CaCl₂ procedure (28,29). Recombinant phage were grown in JM103, and single-stranded phage DNA was isolated by slight modifications of published procedures (28,29). DNA sequencing by the dideoxynucleotide chain termination procedure was performed as described (28-30), except reactions were incubated at 37°C. The Klenow fragment of DNA polymerase I was purchased from Boehringer-Mannheim, New England Nuclear Corp. or BRL with approximately equivalent results. 8% polyacrylamide gels (31), either 36 or 90 cm in length, were used. Gels were soaked 5-10 min. in 10% acetic acid, dried on a gel dryer, and exposed to X-ray film.

RESULTS

Rearranged J κ but no C κ genes were detected in LCLs from the patient.

In DNA blotting experiments, the V κ , J κ , and C κ genes appeared normal in

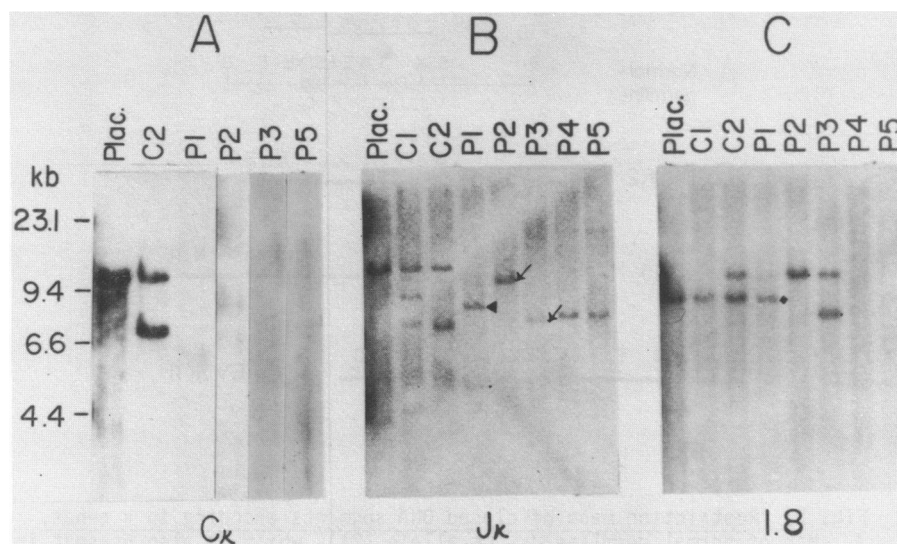


Fig. 1. Blot of BamHI-digested genomic DNA from LCLs and from normal placenta annealed with Ig κ chain probes. Lanes contained 10 μ g of cellular DNA from: Plac. - normal placenta; C1-C2, LCLs from normal human; P1-P5, LCLs from κ -deficient human. P4 was a cloned cell line; P1-P3 and P5 were uncloned.

The sizes (in kb) of λ bacteriophage DNA digested with HindIII, electrophoresed in a parallel lane, are indicated on the left. Blots were annealed with the probes indicated below each panel. The DNA regions present in the probes are indicated in Fig. 2.

The fragment indicated by \swarrow in B, lane P2, and by a tiny dot in C, lane P2, was cloned into the recombinant λ phage EVJK2. The arrow in B, lane P3, indicates the other J κ fragment that also annealed with the 1.8 kb probe in LCLs from the patient. The \blacktriangleleft in B, lane P1, indicates the Bam fragment a portion of which was cloned in EVJK11 (from a MboI library). The \blacklozenge in C, lane P1, indicates the 8.1 kb Bam fragment cloned in EV15.

non Ig-producing cells from the κ -deficient patient. However, when the nucleotide sequence of the C κ genes from both alleles was determined, it was found that in each of the C κ coding regions a single different point mutation had occurred, resulting in the replacement of highly conserved amino acids responsible for maintaining the secondary structure of the C region with different amino acids. Thus, neither of the C κ genes appeared to encode a functional κ chain (32). To examine the effect of these mutations on the structure of the κ genes in B cells from the patient, B lymphoblastoid cell lines (LCLs) were produced by transformation of the patient's blood leukocytes with Epstein-Bar virus (19). Southern blotting experiments on DNA from a few cell lines from the patient, including one cloned cell line,

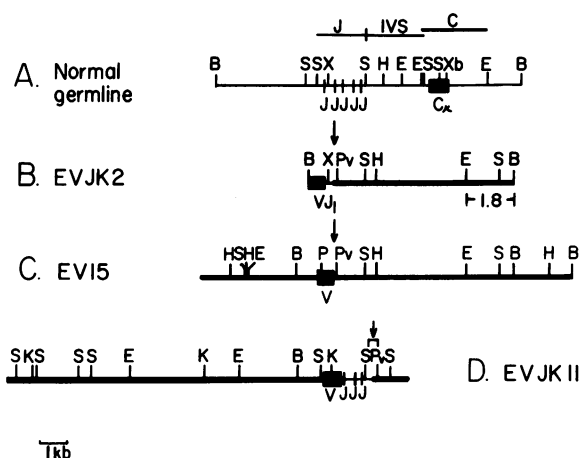


Fig. 2. Restriction maps of cloned DNA segments encoding Ig κ genes.

A. Map of normal germline J κ -C κ allele (21), which is also present in non Ig-producing cells from the κ -deficient individual. Above the map are indicated the DNA segments used as J κ , IVS, and C κ probes.

B,C. EVJK2 contains a misaligned recombined VJ κ 1 segment located 5' to the site of recombination with the EV15 fragment. The maps of EVJK2 and EV15 are identical 3' to the vertical arrow, where the 3' flank of the EV15 V κ gene has recombined with the 5' flank of J κ 2.

D. The EVJK11 fragment contains a misaligned recombined VJ κ 3 segment located 5' to the site of recombination (at arrow) with an unidentified DNA segment.

Maps of cloned DNA segments were obtained utilizing the technique of "in gel" hybridization (47).

Code for restriction enzymes: B, BamHI; E, EcoRI; H, HindIII; K, KpnI; Pv, PvuII; P, PstI; S, SacI (SstI); Xb, XbaI; X, XhoI.

indicated that the LCLs contained rearranged J κ fragments which did not anneal with the C κ probe, and that no germline J κ -C κ gene segments were present (Fig. 1A,B). The probes utilized are indicated on the map in Fig. 2A. The absence of C κ but not of J κ gene segments can be expected from the fact that the patient's B cells express λ chains (1; M. Cooper and A. Solomon, personal communications), since λ + B cells have more frequently deleted C κ than J κ gene segments (2,3,9,11).

Analysis of an aberrantly recombined V-J κ fragment from the patient.

To characterize the recombination events which produced rearranged J κ fragments lacking associated C κ gene segments, one such fragment (the 7.6 kb BamHI fragment indicated with an arrow in Fig. 1B, lane P3) was cloned into Bam arms of the λ bacteriophage vector, L47.1 (26). The cloned fragment (EVJK2) hybridized with nick-translated probes for human V κ (HK101) (12) and J κ gene segments, but not with probes for the IVS between J κ and C κ or for

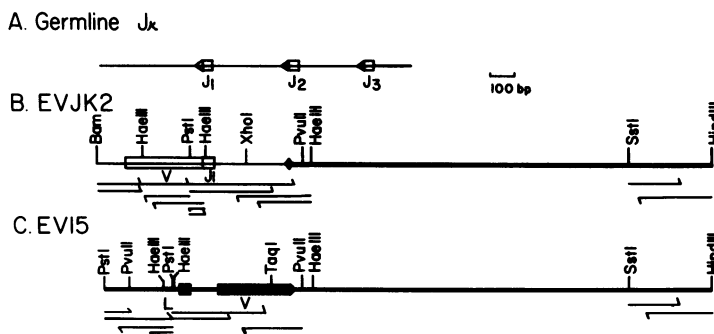


Fig. 3. Sequencing strategy for EVJK2 and EV15 fragments. Restriction maps of a portion of the germline J κ locus (A) aligned with EVJK2 (B) to illustrate the site of recombination in EVJK2 between the 5' border of J κ 2 and the 3' border of the V κ gene in EV15 (C).

Each arrow indicates direction and length of an independent sequencing reaction, usually performed on different M13 clones.

Although no fragment which overlapped the PstI site located 5' to the leader peptide of the EV15 V κ gene was sequenced, restriction enzyme mapping data agreed completely with the sequence. For example, the 57 bp HaeIII fragment containing this PstI site was observed.

the C κ gene segment (22). The map of the EVJK2 fragment, with the location of the V and J gene segments indicated, is given in Fig. 2B. The map showed that 3' to the recombined V-J gene segments an additional recombination had occurred, resulting in the displacement of the C κ gene segment from the recombined V-J segments.

The nucleotide sequence of the region in EVJK2 which hybridized with the V κ and J κ probes was determined by cloning restriction fragments into M13 phage vectors (28,33), and sequencing by the dideoxynucleotide procedure (28-30), according to the strategy indicated by the arrows under the restriction map (Fig. 3B). The sequence (Fig. 4) indicated that the 5' end of the Bam fragment encoded a V κ protein 95% homologous to the amino acid sequence of the subgroup 4 V κ protein, LEN (13). However, the V κ gene has recombined with the J κ 1 gene segment incorrectly, resulting in a frameshift mutation, which altered the amino acid sequence encoded by the J κ 1 gene segment. Although the frameshift mutation did not create a nonsense mutation in framework 3 (FR 3), the amino acid sequence differs at each amino acid position from the normal J κ 1-encoded sequence. It is not known whether such a κ chain could interact effectively with heavy (H) chains to form a functional antibody molecule. The nucleotide sequence of the region 3' to J κ 1 was identical to the sequence of the normal germline J κ -C κ segment (23),


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BamHI
GGATCCAATTATCTGCTGACTTATAATACTACTAGAAAGCAAAATTTAAATGACATATTTTC
      10      20      30      40      50      60

AATTATATCTGAGACAGCGTGTATTAAGTTTATGTATTAATCATTGTCCATTACTGACTACA
      70      80      90     100     110     120

-4  A A Y Q D I V M T Q S P D S L A V S L Q
   130 140 150 160 170 180
9  TGCCTACGGGACATCGTATGACCCAGTCTCCAGACTCCCTGGCTGTCTCTGGGC
      130 140 150 160 170 180

      23 24 CDR 1
E R A T I N C N* A S Q S V L Y S S N* K
190 200 210 220 230 240
GAGAGGGCCACCATCAACTGCAATGCCAGCCAGAGTGTATATACAGCTCCAACAATAAG
      190 200 210 220 230 240

      34 35 FR 2
N Y L A W Y Q Q K P G Q P P K L L I Y W
250 260 270 280 290 300
AACTACTTAAGTTGGTACCAGCAGAAACCAAGGACAGCTCCTAAGCTGCTCATTACTGG
      250 260 270 280 290 300

CDR 2      56 57 FR 3
A S T R E S G V P D R F S G S G S G T D
310 320 330 340 350 360
GCATCTCCGGGAATCCGGGGTCCCTGACCGATTCACTGGCAGCGGTCTGGGACAGAT
      310 320 330 340 350 360

      PstI
F T L T I S S L Q A E D V A V Y Y C Q Q
370 380 390 400 410 420
TTCACCTCTCACCATCAGCAGCTCCAGGCTGAAGATGTGGCAGTTTATTACTGTGCAACAA
      370 380 390 400 410 420

CDR 3      95 Jk1
Y Y S T L N G R S A K Q P R W K S N
430 440 450 460 470 480
TATTATACTCTCCCTGGACGTTTGGCCAAAGGACCAAGGTGAAATCAAACTGTAGT
      430 440 450 460 470 480

AGAATTTAAACTTTGCTTCTCAGTTGTCTGTCTCTCTGTTCCCTGTGTCTATGAAGTG
490 500 510 520 530 540

      G
ATCTATAAGCTGACTCTGCAATCAGCCTCTGATATCCCTCAAGGAAAAGATAAAGATAAG
550 560 570 580 590 600

      XhoI
TCTGTAGTCAAACTCGAATGATTGACATTTTCTTTGAAGAGCAAGCAAGATTCATG
610 620 630 640 650 660
      GT

CATTGGGTGAGAACTTGTCTAACTAATAGCTTCAGAAATGTCTCTGGGAAACATAACA
670 680 690 700 710 720

      A
TGTTCTGGACAGAGCCTTGGTCAATTGTCAAGAAAGGAGTTTTGTATAGGAGGGAAGTT
730 740 750 760 770 780

      Recombination site
AAGAGGAACATTGTGACAGTGTATACACCCCTGTTACAAAAACCTCAAGTTCTCTCAGT
790 800 810 820 830 840

      PvuII
GGGATTGCCTCAGCTGCTGCTGAAAATGTTTCAAGATGTGGAACCTGGGTGGCC
850 860 870 880 890

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Fig. 4. Sequence of EVJK2: recombined V κ 1 segments and site of recombination with EV15. The site of recombination with the 3' flank of the V κ gene in EV15 occurred after nucleotide 796, precisely at the 3' end of the heptamer recognition sequence 5' to J κ 2. The heptamer and nonamer recognition sequences are boxed.

A frameshift mutation has occurred at the V κ 1 junction, putting the J κ 1 gene out of correct reading frame. The protein sequence encoded is indicated. At nucleotide positions 538 and 573 the sequence disagrees with ref. 23, but agrees with ref. 14. At position 550 the EVJK2 sequence disagrees with the published sequences (14,23). Their determination is given above the EVJK2 sequence. At positions 659, 660, 708 only one sequence has been published (23) and it disagrees with EVJK2. RNA splice sites are indicated by: /. Amino acids which differ from the subgroup 4 protein, LEN, are indicated with asterisks.

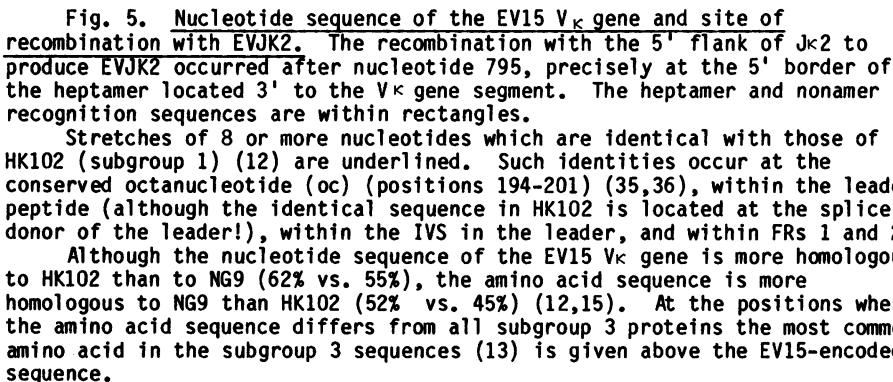
except for 6 nucleotides at scattered positions (at two of these positions the sequence agreed with the sequence of the normal J κ 1 flank published by Klobeck et al., 14), until the site of the second recombination on this

allele at the 5' border of J κ 2. The sequence of the recombined DNA appeared identical with the 3' flanking regions of human V κ genes, as it contained the conserved heptamer and nonamer of the V-J joining sequences, separated by a 12 bp spacer.

Cloning and sequencing of the V κ gene whose flanking sequences are in EVJK2.

To determine whether the DNA which had recombined with the J κ 2 segment in the patient's LCLs encoded a V κ gene, the non-rearranged form (8.1 kb Bam fragment) of this DNA segment was cloned from the LCL derived from the patient analyzed in Fig. 1C, lane P1 (8.1 kb fragment is marked with a diamond). The 1.8 kb EcoRI-Bam fragment from EVJK2 (Fig. 2B) was used as a probe to screen the recombinant λ bacteriophage. The map of the fragment obtained, EV15 (Fig. 2C), is identical to the map of the DNA segment which anneals with the 1.8 kb probe in non Ig-producing cells, e.g. placenta (data not shown). The maps of EV15 and EVJK2 in the region located 3' to the recombination site (indicated by the arrows) are identical (Fig. 2B,C), and the sequences of the 340 bp SstI-HindIII fragments located 1.4 kb 3' to the site of recombination are identical in EVJK2 and EV15 (data not shown). The nucleotide sequence of a 854 bp region in EV15 containing the recombination site was determined, according to the strategy indicated in Fig. 3C, and is presented in Fig. 5.

By a computer aided search, the region of EV15 located immediately 5' to the recombination site was found to contain a V κ gene which is very different from all previously identified V κ genes (12-17). Although the EV15 V κ gene contains no obvious mutations which would prevent it from producing a functional κ chain, none of the large number of κ chains and κ cDNAs which have been sequenced (12-17) appear to be encoded by this V κ gene. The protein sequence encoded by EV15 is most closely related to subgroup 3 V κ proteins, 52% homologous to the most closely related member of this group (encoded by the cDNA clone, NG9 (15), and 45-49% homologous to subgroup 1,2 and 4 V κ proteins (12,13). However, the nucleotide sequence of the EV15 V domain is more related to the subgroup 1 V κ gene, HK102, than to NG9 (62% as compared to 55% homology). At 33% of the amino acid residues the sequence of the EV15 V κ protein differs from all the amino acids at that position in published sequences of subgroup 3 proteins. The most common amino acid present at these positions in subgroup 3 V κ proteins is indicated above the EV15 amino acid sequence in Fig. 5 (13). Because the differences between the EV15 sequence and other V κ sequences are so great, it appears unlikely that the EV15 V κ sequence was derived by somatic mutation of a V κ gene belonging to



one of the known subgroups.

Although the sequence of the EV15 V κ gene differs greatly from that of other human V κ genes, the overall features of the gene are in accord with those of other V κ genes. The signal peptide is 20 amino acids in length, rather than the more common 22, but it is very hydrophobic and has a leucine-rich region, which is typical of Ig V signal sequences. Furthermore, Pecht et al. (17) have also identified a human V κ gene with a signal peptide 20 amino acids in length. The V κ domain encoded by the EV15 V κ gene is of the appropriate length (95 amino acid residues), and many of the conserved amino acids of V κ proteins are present (13) (including the invariant cysteines, positions 23 and 88, and tryptophan, position 35, required to form a stable intradomain disulfide bond) (34). Both the donor and acceptor RNA splice sites within the codon for asp (D) at the -4 position within the signal peptide differ by one nucleotide from the canonical sequences (35): the donor site, at nucleotide position 352, is TG/GT instead of AG/GT, and the acceptor, at nucleotide position 497, is CAG/A instead of CAG/G. But, as these sequences are present in functional splice donors and acceptors (35), presumably this IVS can be correctly spliced. Furthermore, the conserved octanucleotide, the 5' end of which is located approximately 100 bp 5' to the initiator met of all V κ and V λ genes (36,37) is located 110 nucleotides 5' to the initiator met (positions 194-201).

A comparison of the nucleotide sequences of EV15 and EVJK2 locates the precise site of recombination between the EV15 fragment and the 5' flank of J κ 2 which produced the EVJK2 fragment. The sequence of the region located 3' to nucleotide 795 in the EV15 sequence (Fig. 5) is identical to the sequence of EVJK2 3' to nucleotide 796 (Fig. 4), indicating that the site of recombination is precisely at the 5' end of the heptamer at the 3' flank of the EV15 V κ gene, and at the 3' end of the heptamer located at the 5' border of J κ 2. All other such reciprocal recombination products which have been described have also been derived by recombinations which occurred precisely at the 5' end of the V and 3' end of the J κ heptamers (6-8), or precisely at the 5' end of the D $_H$ and 3' end of the J $_H$ heptamers (38).

The EV15 V κ gene is a member of a single gene family.

Since the sequence of the EV15 V κ gene differs so greatly from all other known human V κ sequences, it appears to not be a member of any of the previously identified V κ subgroups. To determine if the EV15 V κ gene is related to other genes in the human genome, as would be expected if it were a member of a typical V κ gene family, the 572 bp PstI-PvuII fragment encoding

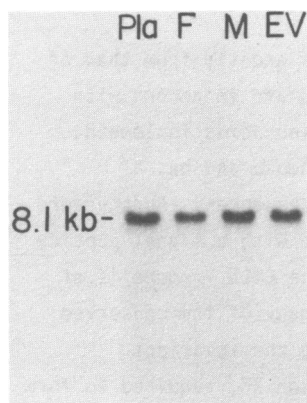


Fig. 6. Blot of DNAs from non Ig producing tissues annealed with the EV15 V κ gene. The lanes contained BamHI-digested DNA from: Pla, normal placenta; F, father of patient; M, mother of patient; EV, patient. The blot was annealed with the 572 bp PstI-PvuII fragment encoding the V κ domain of EV15.

the EV15 V κ region was annealed to a blot containing DNA from normal placenta and from blood leukocytes from the κ -deficient individual and his two parents. One fragment was detected in all four of the DNAs digested with either Bam (Fig. 6) or EcoRI (not shown). The fact that the EV15 V κ gene is not a member of a multi-gene family further indicates that the differences between the EV15 V κ gene and other V κ genes are not due to somatic mutation, because if EV15 were derived by extensive somatic mutation from a member of the previously identified V κ subgroups, it should anneal to other members of the subgroup and it should not anneal so strongly to a single DNA fragment from non Ig-producing cells. The existence of a V gene which is not a member of a group of related genes has been described before, as the V κ gene expressed in the mouse myeloma, MOPC 167, is also the only member of a V κ group (39,40).

The EV15 V κ gene is frequently rearranged in LCLs from both the normal and κ -deficient individual.

Since the EV15 V κ gene has not been found to be expressed we decided to determine how commonly it was rearranged in LCLs from the κ -deficient and normal humans. To search for possible reciprocal recombination products, as was found in EVJK2, the 1.8 kb EcoRI-Bam fragment from the region 3' to the site of recombination (Fig. 2B) was used to probe blots of DNA from normal placenta and from LCLs from the patient and from a normal individual. The 1.8 kb probe annealed with a unique Bam fragment, 8.1 kb in length in placenta DNA (Fig. 1C, lane Plac.), which was rearranged in one of two LCLs from the normal individual (Fig. 1C, lane C2) and in three LCLs from the κ -deficient individual (lanes P1-P3). In one uncloned (P5) and one cloned (P4) LCL derived from the patient no fragments were detected by the 1.8 kb

probe. In the two control LCLs none of the three rearranged J_K fragments co-migrated with fragments that annealed with the 1.8 kb probe (Fig.1B,C: lanes C1,C2), whereas in three LCLs from the patient, two of the five rearranged J_K fragments co-migrated with fragments that annealed with the 1.8 kb probe (using two different enzymes). The J_K Bam fragments from the patient's LCLs which also annealed with the 1.8 kb probe are indicated by arrows in lanes P2 and P3 (Fig. 1B). If the EV15 flanking sequences had recombined with the J_{K1} flanking sequences in the three other rearrangements, the J_K probe would not detect this fragment, since it only detects 14 nucleotides 5' to the J_{K1} flank (23). Although one of the control LCLs (lane C2) contained DNA from cells which were mostly λ^+ (by RNA blotting experiments; data not shown), the sample number is too low to conclude that the recombination between this fragment and J_K segments was specific to the patient's LCLs.

To determine which chromosome encoded the EV15 V_K gene, a blot of DNAs from human-mouse somatic cell hybrids which contained partial complements of the human chromosome set (41) was probed with the 1.8 kb probe. The fragment was localized to the short arm of chromosome 2, in the sub-region containing the κ chain genes (42,43) (data not shown).

Cloning of a second aberrant V-J fragment from the κ -deficient individual.

A second example of a recombined V- J_K allele which also lacked a C_K gene was cloned from a LCL derived from the patient by screening the MboI DNA library described above with the J_K probe. The fragment which was cloned (EVJK11) was derived from the 8 kb Bam fragment present in the DNA analyzed

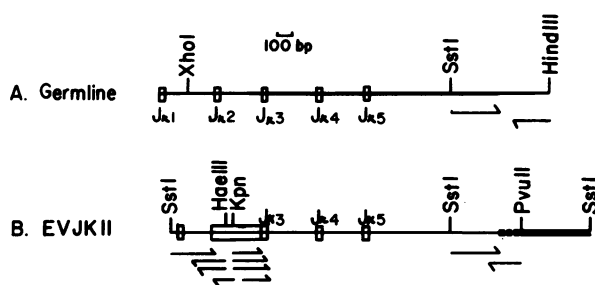


Fig. 7. Alignment of EVJK11 and the germline J_K segments; sequencing strategy for EVJK11. The site of recombination which deleted the C_K gene from EVJK11 occurred within the SstI-HindIII fragment located 3' to the J_K gene segments, within the region indicated by the bracket (determined by partial nucleotide sequencing and by restriction enzyme mapping; data not shown).

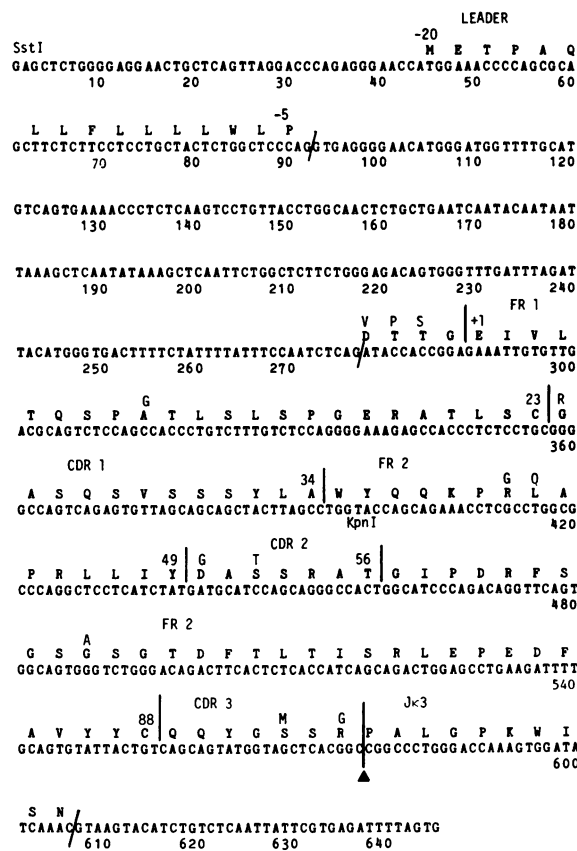


Fig. 8. Sequence of the recombined V κ 3 segment of EVJK11. The EVJK11 V κ gene is a member of subgroup 3. The amino acids which differ from the most closely related member of subgroup 3, NG9, are given above the EVJK11 amino acid sequence (15). At the site of recombination between the V and J κ 3 gene segments, indicated by \downarrow , 8 nucleotides from the 5' end of J κ 3 have been deleted, creating a frameshift mutation.

in Fig. 1B, lane P1 (marked with a triangle). The recombination which separated the C gene from this V-J gene segment occurred at a different site from that in EVJK2, within the IVS located 3' to the J κ genes (indicated by the arrow above Fig. 2D), and did not involve the EV15 fragment (data not shown). We have not determined the source of the DNA which has recombined with the IVS in EVJK11. If it is derived from the region located 3' to the C κ gene, this recombination would result in deletion of the C κ gene segment from the chromosome. By probing Southern blots of LCLs with the 0.9 kb SstI fragment which includes the site of recombination, we have found that this

fragment is commonly rearranged in LCLs from the κ -deficient and from the normal human (data not shown).

The nucleotide sequence of the region encoding the recombined V-J gene segment was determined according to the strategy given in Fig. 7. The sequence demonstrated that the EVJK11 fragment contained a subgroup 3 V κ gene which had recombined with the J κ 3 gene segment (Fig. 8). As in EVJK2, the recombination was aberrant, resulting in a deletion of at least 8 nucleotides from the 5' end of J κ 3 and a frameshift so that the J κ 3 coding triplets were out of reading frame, but again, no termination codon was created in the J κ 3 segment. Although the amino acid sequence of the resulting FR3 sequence differs greatly from the normal sequence encoded by J κ 3, we do not know if this would prevent the formation of a functional κ chain. The J κ 3 gene segment did not contain any other nucleotides which differed from the published germline sequence (23).

DISCUSSION

Different types of recombination events can result in displacement of a recombined V-J κ gene segment from the C κ locus.

Although the two recombination events described above, resulting in separation of C κ genes from recombined V-J κ gene segments occurred in B cell lines derived from a κ -deficient individual, we do not know if these recombinations are causally related to the mutations within the C κ genes of this κ -deficient individual. Both of the V-J κ segments whose associated C κ genes had been deleted had undergone an incorrect V-J recombination, resulting in V κ proteins with unusual FR4 regions. Since no κ chains with such altered FR4 regions have been previously reported, it appears likely that such κ chains would not be able to associate effectively into antibody molecules. Thus, the recombinations which resulted in removal of the recombined V-J segments from the C κ locus may have been activated by the misaligned V-J recombination or by the mutated C κ genes.

The recombination events that generated the EVJK2 and EVJK11 fragments would have resulted in the separation of a recombined V-J segment from the κ transcription activator (44-46) and the C κ gene. Two possible pathways for the generation of EVJK2 are diagrammed in Fig. 9. Since the LCLs from which the EVJK2 fragment was cloned did not contain any C κ genes, an additional recombination event at this allele would be required to delete the C κ gene. Alternatively, if the EV15 V κ gene were located 3' to the C κ gene, interchromatid recombination would have resulted in deletion of the C κ gene.

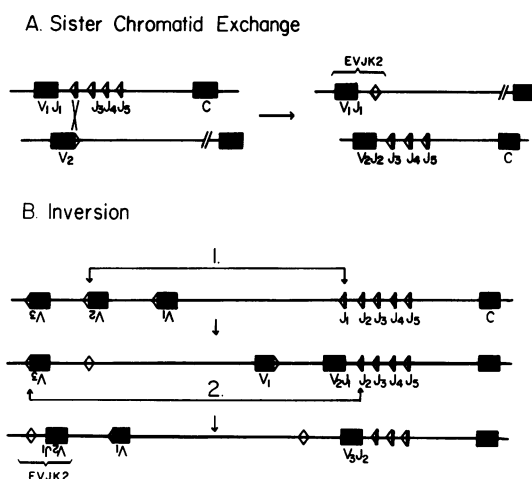


Fig. 9. Diagram of recombination events which could produce the EVJK2 fragment. The EVJK2 fragment is indicated by brackets above the top line in A and below the bottom line in B.

A. Generation of EVJK2 by sister chromatid exchange (interchromatid recombination) subsequent to V1-J1 recombination. The order of recombination could have been reversed, i.e. joining of the V κ and J κ 2 flanking sequences could have occurred prior to the V-J κ 1 recombination. However, if recombinations occurred in this order, the reciprocal fragment bearing a J κ 1 segment would be separated from the C κ gene and the κ enhancer, and this conceivably could eliminate the recombinatorial activity of this locus. An additional recombination would be required to delete the C κ gene unless the EV15 κ gene were located 3' to the C κ gene.

B. Generation of EVJK2 by inversion. (1) Production of a recombined V2-J1 fragment. (2) A second inversion event forms the recombined V3-J2 fragment and simultaneously produces the V2-J1 fragment (EVJK2) separate from the C κ gene, although still linked on the same chromosome. An additional recombination would be required to delete the C κ gene segment.

Data have been presented suggesting that C κ genes are frequently deleted from mouse and human λ + cell lines by recombination between a specific sequence within the IVS between the J κ and C κ gene segments and a specific DNA sequence (RS) originating from the region 3' to the C κ genes (2,3). Although it was not determined whether the RS DNA did indeed originate from 3' to the C κ gene, Durdick et al. (2) did find that it originated from the chromosome encoding the κ chain genes. It is possible, albeit unlikely, that the recombination events these authors studied separated the V-J κ gene segment from the C κ gene segment, but did not actually result in deletion of the C κ gene from the chromosome. One of the recombination events which resulted in displacement of a recombined V-J κ segment from a C κ gene in an LCL from the κ -deficient human (EVJK11) occurred at a site located near the 5' end of the

IVS between the J κ and C κ gene segments, as did the recombinations studied by Durdick et al. (2) and Siminovitch et al. (3). We have not determined whether the DNA which has recombined with the VJ segment in EVJK11 originated from the κ chain chromosome.

Generation of the EVJK2 fragment.

The existence of DNA fragments integrated into the genome which appear to be reciprocal products of V κ -J κ recombination is consistent with V κ -J κ recombination occurring by interchromatid recombination, by intrachromatid inversion (7), or by re-insertion of deleted DNA segments produced by intrachromatid recombination (8). This is also true for the EVJK2 fragment. The recombined V and J flanking regions located 3' to a recombined VJ κ 1 gene segment could have been produced by interchromatid recombination (Fig. 9A), or by inversion if only some of the V κ genes are inverted with respect to the J genes in germline DNA or if the second VJ recombination event utilized V κ genes located 5' to the region inverted during the first recombination event (Fig. 9B). Finally, the recombined V and J flanks in EVJK2 could have been derived by re-insertion of deleted DNA (not shown) if the excised DNA included the recombined VJ κ 1 gene segment, in addition to the recombined flanking sequences, because the sequence between J κ 1 and J κ 2 is essentially identical to the normal germline sequence, showing no evidence of recombination. It is also possible that V κ and J κ 1 sequences present in excised DNA recombined prior to reinsertion of an excised fragment, perhaps simply because V and J flanking sequences are recombinogenic.

The structure of the EVJK2 fragment proves that more than one V-J recombination event can occur at one J κ -C κ allele, as has been previously hypothesized (5). Two events were required to produce the recombined V-J κ 1 gene segments and the recombined V-J κ 2 flanking sequences; presumably a third event (perhaps of the type which occurred in EVJK11) resulted in deletion of the C κ gene from the chromosome.

It is attractive to postulate that recombinations causing the displacement of the recombined V-J segments from the κ enhancer and C κ gene segment would have the function of allowing replacement of a non-functional V-J segment with another recombined V-J fragment which, perhaps, will be functional, i.e. able to encode a κ chain which can effectively recombine with the H chain synthesized in that cell. Since in the κ -deficient human no κ gene can be functional, recombination events at the κ locus apparently continue, until the C κ genes are deleted. Since recombination events which may have deleted the C κ gene from λ -producing cell lines occurred at a

heptamer identical with the heptamer present in V-J recombination signals, the deletion of the C κ gene segment may also involve the V-J recombination machinery (2,3).

A new human V κ gene identified.

The finding of a human V κ gene which does not belong to any of the four known human V κ subgroups and which has never been found to be expressed was surprising because significant portions of more than 85 expressed V κ domains have been sequenced and they all belong to one of the four subgroups (12-17). Furthermore, by using the 1.8 kb fragment from EVJK2, which corresponds to the region located 3' to the EV15 V gene, to probe blots of LCL DNA, we found that this fragment was rearranged in, or deleted from, one of the two LCLs examined from a normal human and in all of the LCLs from the κ -deficient individual (Fig. 1C). Although this V κ gene is frequently rearranged in the LCLs we have examined, we do not know whether it is ever functionally rearranged. To begin to understand the function of this V κ gene it will be important to examine its structure in B cells from a normal human and to determine if it is transcribed into κ chain mRNA in these cells. To shed light on why it is so frequently rearranged in the LCLs we studied, it will be important to determine where it is located relative to other Ig V κ gene segments and to the C κ gene segment.

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Abbreviations.

C, constant; CDR, complementarity-determining region; FR, framework region; H, heavy; Ig, immunoglobulin; IVS, intervening sequence; LCL, lymphoblastoid cell line; V, variable.

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